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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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09/839,658

04/19/2001

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S2037-700210

9914

37462 7590 06/01/2009

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EXAMINER

STRZELECKA, TERESA E

ART UNIT

PAPER NUMBER

1637

NOTIFICATION DATE

DELIVERY MODE

06/01/2009

ELECTRONIC

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UNITED STATES PATENT AND TRADEMARK OFFICE

**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Ex parte ALLAN BRADLEY and WEI-WEN CAI

Appeal 2009-001905¹
Application 09/839,658
Technology Center 1600

Decided:² May 28, 2009

Before DONALD E. ADAMS, RICHARD M. LEBOVITZ, and
FRANCISCO C. PRATS, *Administrative Patent Judges*.

PRATS, *Administrative Patent Judge*.

¹ Baylor College of Medicine is the real party in interest (App. Br. 3 (filed May 5, 2008)).

² The two-month time period for filing an appeal or commencing a civil action, as recited in 37 C.F.R. § 1.304, begins to run from the decided date shown on this page of the decision. The time period does not run from the Mail Date (paper delivery) or Notification Date (electronic delivery).

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 involving claims to a method of generating a molecular profile of genomic DNA by hybridization. The Examiner has rejected the claims as obvious. We have jurisdiction under 35 U.S.C. § 6(b).

We affirm.

STATEMENT OF THE CASE

Claims 1-14, 17, 67, 68, and 72 stand rejected and are on appeal (App. Br. 3). Claim 1 is representative and reads as follows:

1. A method for generating a molecular profile of genomic DNA by hybridization of a genomic DNA target to a plurality of immobilized nucleic acid probes, wherein the plurality is a collection of clones that represent all of a chromosome or a genome of an organism, the method comprising:

(a) providing the plurality of nucleic acid probes comprising a plurality of immobilized nucleic acid segments in an array with each probe at a known location, wherein each probe is a member of a genomic library cloned in a vector and each probe in the vector having a cloned nucleic acid insert greater than 50 kilobases, wherein the plurality of probes represents all of the chromosome or the genome;

(b) contacting the immobilized probes with a sample of target nucleic acid comprising fragments of genomic nucleic acid,

wherein said fragments include both strands of a double-stranded genomic DNA fragment and include at least 30% repetitive sequences, and

wherein both strands are labeled with a detectable moiety, wherein each labeled fragment consists of a length smaller than 200 bases, and the contacting is under conditions allowing specific hybridization of both strands of the labeled fragment of the target nucleic acid to the probe nucleic acid; and

(c) observing an amount and location of labeled genomic nucleic acid hybridized to each immobilized probe, to detect regions of amplification or deletion in the sample, wherein positional information of clones on the arrays and chromosomes is correlated,

wherein said method results in less aggregating hybridization to said probes relative to hybridization of said target genomic nucleic acid to said probes using target nucleic acids with labeled fragments of length greater than 200 bases, or said method results in less background relative to hybridization of said target genomic nucleic acid using target nucleic acids with labeled fragments of length greater than 200 bases,

thereby generating a molecular profile of the chromosome or genome of the sample genomic nucleic acid.

The Examiner cites the following documents as evidence of unpatentability:

Waggoner et al.	US 5,268,486	Dec. 7, 1993
McGill et al.	US 5,658,730	Aug. 19, 1997
Kallioniemi et al.	US 2002/0132246 A1	Sep. 19, 2002

J.R. Pollack et al., *Genome-wide analysis of DNA copy-number changes using cDNA microarrays*, 23 NATURE GENETICS 41-46 (1999).

J. Mackey et al., *Use of Random Primer Extension for Concurrent Amplification and Nonradioactive Labeling of Nucleic Acids*, 212 ANAL. BIOCHEM. 428-435 (1993).

S. Anderson, *Shotgun DNA sequencing using cloned DNase I-generated fragments*, 9 NUCL. ACIDS RES. 3015-3027 (1981).

C.P. Ordahl, *Sheared DNA fragment sizing: comparison of techniques*, 3 NUCL. ACIDS RES. 2985-2999 (1976).

GibcoBRL Catalog (pages 18-15 and 18-16, 1995-96).

The following rejections are before us for review:

Claims 1-6, 12-14, 17, 67, 68, and 72 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, and Mackey (Ans. 4-8).³

Claims 7, 8, and 10 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, Mackey, and Anderson (Ans. 8-9).

Claim 9 stands rejected under 35 U.S.C. § 103(a) as unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, Mackey, Anderson, and Waggoner (Ans. 9-10).

Claim 11 stands rejected under 35 U.S.C. § 103(a) as unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, Mackey, Anderson, and Ordahl (Ans. 10-12).

OBVIOUSNESS -- CLAIMS 1-6, 12-14, 17, 67, 68, and 72
ISSUE

The Examiner finds that Kallioniemi discloses a process having the basic steps recited in claim 1, the reference differing from claim 1 in not using DNA sample fragments of less than 200 base pairs (*see* Ans. 4-6). To meet that limitation, the Examiner cites McGill as disclosing a hybridization process that uses probes of 10-500 base pairs, “with the optimal probe sequence being about 20 bases” (*id.* at 7).

Based on these teachings, the Examiner concludes that a person of ordinary skill in the art would have considered it obvious “to have used short target fragments of McGill et al. in the hybridization method of Kallioniemi

³ Examiner’s Answer mailed June 24, 2008.

et al. The motivation to do so, provided by McGill et al., would have been that fragments with about 20 bases allow formation of duplexes which are stable and selective” (*id.* (citing McGill, col. 6, ll. 1-3)).

The Examiner urges that Pollack provides further motivation for using short fragments in Kallioniemi’s hybridization process because Pollack discloses “that reducing the size of genomic DNA before labeling improved labeling efficiency by providing greater accessibility of the DNA template following digestion. Such greater accessibility would also allow more specific annealing of the probes to the array” (Ans. 7).

The Examiner further concedes that Kallioniemi and McGill differ from claim 1 in that neither reference “specifically teach[es] double-stranded DNA fragments labeled on both strands” (*id.* at 8). To meet that limitation, the Examiner cites Pollack as disclosing a process of labeling digested genomic DNA with a “BioPrime labeling kit from GibcoBRL [which] . . . contains a DNA polymerase and random primers . . . [;] therefore, the labeling reaction would result in amplified double-stranded DNA with both strands labeled” (*id.* at 8 (citing GibcoBRL catalog, p. 18-16)).

Based on these teachings, the Examiner concludes that a person of ordinary skill in the art would have considered it obvious to use GibcoBRL’s BioPrime labeling kit “to produce double-stranded labeled DNA fragments in the method of Kallioniemi et al., McGill et al. and Pollack” (Ans. 8). The Examiner reasons that “[t]he motivation to do so is provided by Mackey et al., who teach using the BioPrime kit to label genomic DNA to create probes for hybridization, and teach that probes were prepared from as little as 1 ng of starting material” (*id.*).

Appellants argue the claims subject to this rejection together, “with claim 1 being representative” (App. Br. 7). The thrust of Appellants’ position is that the “rejection is improper because no proper *prima facie* case of obviousness has been established” (*id.* at 8; *see also* Reply Br. 8-11).

Specifically, Appellants argue, “[o]ne skilled in the art would not have been motivated to modify Kallioniemi or to combine Kallioniemi with any of the [other cited references]” (App. Br. 9). Moreover, Appellants urge, “Kallioniemi, either alone or [in] combination with the secondary citations, does not teach or suggest all the claim elements of claim 1” (App. Br. 11).

In particular, Appellants argue that Kallioniemi fails to meet claim 1’s requirements of using an array of clones representing all of a chromosome or genome, fails to disclose the claimed “observing” step, and fails to teach or suggest the claimed use of nucleic acid sample fragments of less than 200 base pairs (*id.*). Appellants further argue that the remaining references fail to remedy Kallioniemi’s shortcomings, and that “the Examiner has not properly articulated a finding that there was some teaching, suggestion, or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art to modify the references” (*id.* at 9-10 (citing MPEP § 2143)).

In view of the positions advanced by Appellants and the Examiner, the issues with respect to this rejection are (a) whether the Examiner erred in finding that the cited references teach or suggest all of the limitations of claim 1, and (b) whether the Examiner erred in finding that a person of ordinary skill in the art would have been prompted to provide the samples in Kallioniemi’s hybridization assay with a fragment size of less than 200 base

pairs, and with detectable label on both strands of the sample, as recited in claim 1.

FINDINGS OF FACT ("FF")

1. Kallioniemi discloses "a method of analyzing gene amplification in a tissue specimen by screening multiple genes in a tissue specimen with a genosensor comparative genomic hybridization (gCGH) array that detects which genes are amplified in the tissue specimen" (Kallioniemi [0012]).

2. Kallioniemi discloses:

In Comparative Genomic Hybridization (CGH), DNA from a sample tissue, such as a tumor, is compared to normal human DNA. . . . [T]his is accomplished by labeling the sample DNA with a fluorescent dye, and the reference ("normal") DNA with a fluorescent dye of a different color. Both DNAs are then mixed in equal amounts and hybridized to a DNA chip.

(Kallioniemi [0152].)

3. Kallioniemi discloses that the relative amounts of DNA are quantified as follows:

After hybridization, a multi-color imaging system determines the ratio of colors (for example green to red fluorescence) for each of the probe spots in the array. If there is no difference between the sample DNA and the normal DNA, then all spots should have an equal mixture of red and green fluorescence, resulting in a yellow color. A shift toward green or red for a given spot would indicate that either more green or more red labeled DNA was bound to the chip by that probe sequence. This color shift indicates a difference between the sample and the reference DNA for that particular region on the human genome, pointing either toward amplification or deletion of a specific sequence or gene contained in the clones positioned in the array. Examples of genetic changes that can be detected

include amplifications of genes in cancer, or characteristic deletions in genetic syndromes, such as Cri du chat.

(Kallioniemi [0152].)

4. Kallioniemi discloses that the “color ratio analysis of the genosensor CGH (gCGH) assay has the advantage that absolute quantitation of the amount of a specific sequence in the sample DNA is not required. Instead, only the relative amount compared to the reference (normal) DNA is measured with relatively high accuracy” (Kallioniemi [0154]).

5. Kallioniemi also discloses that, in certain CGH embodiments, the process includes the step of “measuring the amount of nucleic acid test sample hybridized to the candidate genomic regions” (Kallioniemi [0016]).

6. Kallioniemi discloses that the DNA chip or “genosensor” used in its CGH processes “contains an array of large insert DNA clones, each comprising approximately 100,000 nucleotides of human DNA sequence” (Kallioniemi [0152]).

7. Thus, “[s]ince each genetic region to be analyzed needs to be represented on the chip in only 1 or few replicate spots, the genosensor can be designed to scan the total human genome for large deletions or duplications in a single assay” (Kallioniemi [0153]).

8. Kallioniemi discloses that “an array of just 3000 different clones evenly spaced along the human genome would provide a level of resolution that is at least 10 times better than what can be achieved with metaphase hybridization, at a much lower cost and in much less time” (Kallioniemi [0153]).

9. Kallioniemi discloses:

DNA chips may vary significantly in their structure, composition, and intended functionality, but a common feature is usually the small size of the probe array, typically on the order of a square centimeter or less. Such an area is large enough to contain over 2,500 individual probe spots, if each spot has a diameter of 0.1 mm and spots are separated by 0.1 mm from each other. A two-fold reduction in spot diameter and separation can allow for 10,000 such spots in the same array, and an additional halving of these dimensions would allow for 40,000 spots. Using microfabrication technologies, such as photolithography, pioneered by the computer industry, spot sizes of less than 0.01 mm are feasible, potentially, providing for over a quarter of a million different probe sites.

(Kallioniemi [0149].)

10. McGill discloses “probes and methods useful in monitoring the progression of prostate cancer through the use of fluorescence in situ hybridization techniques” (McGill, col. 1, ll. 15-17).

11. Specifically, McGill discloses that “a probe hybridizable to a region of chromosome 8 (8q24.1-24.2) can be used as a prognostic tool for prostate cancer. . . . [I]ncreased copy number in this region of chromosome 8 is indicative of metastatic prostate cancer progression” (*id.* at col. 4, ll. 12-16).

12. McGill discloses that when its probes are hybridized to immobilized nucleic acid samples, oligonucleotide fragments long enough “to provide specific hybridization to a DNA tissue sample, such as a fragment of between about 10 nucleotides to about 20, or to about 30 nucleotides, will find particular utility. Longer sequences, e.g., 40, 50, 100 to 500, even up to full length, are even more preferred for certain embodiments” (McGill, col. 5, ll. 38-43).

13. Regarding suitable sizes for its probes, McGill further discloses:

Nucleic acid molecules having stretches of 10, 20, 30, 50, 60, 65 or even up to and including 100 to 500 nucleotides or so, and those complementary to SEQ ID NO: 1 may also have utility as hybridization probes. These probes will be useful in a variety of hybridization embodiments, such as Southern and Northern blotting in connection with analyzing tissue-specific structural or regulatory genes in tissue samples. The total size of fragment, as well as the size of the complementary stretches, will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the complementary region may be varied, such as between about 10 and about 100 nucleotides, or even full length according to the complementary sequences one wishes to detect.

(McGill, col. 5, ll. 52-67.)

14. Regarding suitable sizes for its probes, McGill further discloses:

The use of a hybridization probe of about 20 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 20 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of particular hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 20 to 30 nucleotides, or even longer where desired.

(McGill, col. 6, ll. 1-10.)

15. McGill discloses that its probes are useful in detecting increased copy number of the relevant sequence in a CGH technique using metaphase chromosome spreads (McGill, col. 13, l. 53, through col. 14, l. 51; *see also* col. 17, ll. 27-42).

16. Pollack discloses “a cDNA microarray-based CGH method, and its application to DNA copy-number variation analysis in breast cancer cell lines and tumours” (Pollack 41). Pollack states that the technique allowed the investigators to “identify gene amplifications and deletions genome-wide and with high resolution, and compare alterations in DNA copy number and gene expression” (*id.*).

17. Pollack discloses that its sample was labeled by “*DpnII*-digest[ing] (New England Biolabs) genomic DNA (2 µg), which was then purified (Qiaquick PCR kit) and random-primer labelled using a Bioprime Labeling kit (Gibco BRL), modified to include in a 100 µl reaction, dATP, dGTP and dTTP (120 µM each), dCTP (60 µM) and Cy5-dCTP or Cy3-dCTP (60 µM)” (Pollack 46).

18. Pollack discloses that “[d]uring optimization of the cDNA microarray CGH procedure, we found that the labelling efficiency was increased by reducing the average fragment size of the genomic DNA before random-primed labelling. This may reflect the increased accessibility of the DNA template following digestion” (Pollack 46).

19. The GibcoBRL Catalog discloses:

The BIOPRIME DNA Labeling System is suitable for biotin labeling of DNA probes for use in nonradioactive Southern blots, Northern blots, plaque lifts, colony hybridizations, and *in situ* hybridizations. The DNA is labeled by random priming in the presence of biotin-14-dCTP. The random priming method requires less template DNA than nick translation and amplifies the template to provide an increased amount of biotinylated probes.

(GibcoBRL Catalog 18-15.)

20. Mackey discloses a “method for efficient nonradioactive labeling of DNA with biotin using random primer extension” (Mackey 428).

21. Mackey discloses:

[T]he random primer biotin labeling system . . . has a number of attractive features. Small amounts of template DNA (as little as 1 ng) can be amplified and labeled resulting in hundreds of nanograms to microgram amounts of biotinylated probe. This amplification method is especially useful for labeling of DNAs which are difficult to isolate in large quantities; these include YACs, cosmids, and DNA isolated from agarose or polyacrylamide gels. The probe size is small and is suitable for *in situ* hybridization procedures.

(Mackey 434.)

PRINCIPLES OF LAW

“During examination, the examiner bears the initial burden of establishing a *prima facie* case of obviousness.” *In re Kumar*, 418 F.3d 1361, 1366 (Fed. Cir. 2005).

As the Supreme Court pointed out in *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007), “a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art.” Rather, the Court stated:

[I]t can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements *in the way the claimed new invention does* . . . because inventions in most, if not all, instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known.

Id. at 418-419 (emphasis added); *see also id.* at 418 (requiring a determination of “whether there was an apparent reason to combine the

known elements *in the fashion claimed* by the patent at issue”) (emphasis added).

Accordingly, as our reviewing court has stated, “obviousness requires a suggestion of all limitations in a claim.” *CFMT, Inc. v. Yieldup Int’l Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (citing *In re Royka*, 490 F.2d 981, 985 (CCPA 1974)).

While holding that some rationale must be supplied for a conclusion of obviousness, the Supreme Court nonetheless rejected a “rigid approach” to the obviousness question, and instead emphasized that “[t]hroughout this Court’s engagement with the question of obviousness, our cases have set forth an expansive and flexible approach” *KSR*, 550 U.S. at 415. The Court also rejected the use of “rigid and mandatory formulas” as being “incompatible with our precedents.” *Id.* at 419; *see also id.* at 421 (“Rigid preventative rules that deny factfinders recourse to common sense, however, are neither necessary under our case law nor consistent with it.”).

The Court thus reasoned that the analysis under 35 U.S.C. § 103 “need not seek out precise teachings directed to the specific subject matter of the challenged claim, for a court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ.” *Id.* at 418; *see also id.* at 421 (“A person of ordinary skill is . . . a person of ordinary creativity, not an automaton.”).

The Court further reasoned that it is obvious to choose from among known solutions to prior art problems:

When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this

leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try might show that it was obvious under § 103.

Id.

ANALYSIS

Appellants' arguments do not persuade us that the Examiner erred in finding that the cited references teach or suggest all of the limitations of claim 1. Nor are we persuaded that the Examiner erred in finding that a person of ordinary skill in the art would have been prompted to provide the samples in Kallioniemi's hybridization assay with a fragment size of less than 200 base pairs, and with detectable label on both strands of the sample, as recited in claim 1.

Appellants argue:

Scanning the human genome using a genosensor *in a single assay*, as disclosed in Kallioniemi, is not the same as a method that uses a plurality of immobilized nucleic acid segments *in an array* that are a collection of clones that represent all of a chromosome or a genome of an organism as defined by claim 1.

(App. Br. 9.)

It may be true that an array-based hybridization process disclosed as a single assay is not necessarily performed using a single array. However, we do not agree that Kallioniemi fails to teach or suggest claim 1's limitation that the sample is contacted with "a plurality of immobilized nucleic acid segments in an array . . . wherein the plurality of probes represents all of the chromosome or the genome."

Specifically, Kallioniemi discloses that its DNA chip, or "genosensor, contains an array of large insert DNA clones, each comprising

approximately 100,000 nucleotides of human DNA sequence” (Kallioniemi [0152] (FF 6)). Thus, “the genosensor can be designed to scan the total human genome for large deletions or duplications in a single assay” (Kallioniemi [0153] (FF 7)).

By stating that “*the* genosensor can be designed to scan the total human genome” (*id.* (emphasis added)), Kallioniemi suggests that a single chip is suitably used to scan the entire genome (*see also* FF8-9). Given these disclosures, we agree with the Examiner that a person of ordinary skill in the art would have reasonably understood that Kallioniemi’s hybridization would use a single array, as required by claim 1.

Nor are we persuaded that Kallioniemi fails to teach or suggest claim 1’s step of “observing an amount and location of labeled genomic nucleic acid hybridized to each immobilized probe.” We note, as Appellants argue, that Kallioniemi determines the relative amount of a particular sequence by simultaneously hybridizing the immobilized probes with a suspected abnormal sample labeled with one dye, as well as a normal sample labeled with a different dye (*see* FF 2-4).

However, claim 1 merely requires “observing *an* amount . . . of labeled nucleic acid hybridized to each immobilized probe” (emphasis added). Claim 1 therefore does not require determining the absolute quantity of the hybridized nucleic acid. Rather, claim 1 encompasses observing relative, or proportional, amounts of the hybridized sample.

Therefore, by determining the amount of the hybridized sample in proportion to the amount of hybridized normal nucleic acid, Kallioniemi does, in fact, observe “an amount” of hybridized nucleic acid -- as compared to other probes in the same array. Moreover, because Kallioniemi explicitly

discloses, in certain CGH embodiments, the suitability of “measuring the amount of nucleic acid test sample hybridized to the candidate genomic regions” (Kallioniemi [0016] (FF 5)), we are not persuaded that the reference fails to suggest observing the amount of nucleic acid hybridized to the array in a CGH procedure.

Nor are we persuaded that the cited references fail to suggest giving Kallioniemi’s labeled nucleic acid samples a size of less than 200 bases. We note that McGill discloses a variety of widely differing size fragments as being suitable for hybridization to immobilized nucleic acids (*see* FF 10-15).

Among the suitable fragment sizes, however, McGill explicitly states that the “use of a hybridization probe of about 20 nucleotides in length allows the formation of a duplex molecule that is both stable and selective” (McGill, col. 6, ll. 1-3 (FF 14)). Moreover, in the context of a cDNA microarray CGH method, Pollack discloses, that “labelling efficiency was increased by reducing the average fragment size of the genomic DNA before random-primed labelling. This may reflect the increased accessibility of the DNA template following digestion” (Pollack 46 (FF 18)).

Given McGill’s disclosure of the suitability of 20 base fragments in hybridization procedures, combined with Pollack’s disclosure that the efficiency of sample labeling for CGH hybridizations is improved by using smaller fragments, we agree with the Examiner that a person of ordinary skill in the art would have been prompted to use fragments of the claimed size in Kallioniemi’s process.

Appellants argue that “[i]t still remains unclear . . . how Mackey and the GibcoBRL Catalog, and therefore Pollack, teach labeling of both strands of genomic DNA. It is also unclear to Appellants how the passage by

Mackey, cited by the Examiner is relevant to teaching labeling of both strands of DNA” (Reply Br. 11).

We are not persuaded that the cited references fail to suggest labeling both strands of Kallioniemi’s nucleic acid fragment samples. As noted above, Pollack discloses using the GibcoBRL BioPrime labeling kit in an array-based CGH procedure (FF 17), and Mackey discloses the kit as being advantageous for labeling nucleic acids used in hybridization procedures (FF 21).

Given the disclosed desirability of using the BioPrime kit for labeling nucleic acid in CGH and other hybridization processes, we agree with the Examiner that a person of ordinary skill in the art would have been prompted to use it in Kallioniemi’s process. Moreover, given the GibcoBRL Catalog’s disclosure that the BioPrime kit labels nucleic acids using random priming in the presence of labeled d-CTP (FF 19), we agree with the Examiner that it was reasonable to find that the random labeling would occur on both strands of the sample.

In sum, we agree with the Examiner that the cited references teach or suggest all of the limitations of claim 1. We also agree that a person of ordinary skill in the art practicing Kallioniemi’s CGH procedures would have been prompted by McGill and Pollack to use nucleic acid fragments of less than 200 bases as the labeled sample, as required by claim 1.

We therefore affirm the Examiner’s rejection of claim 1 under 35 U.S.C. § 103(a) as being unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, and Mackey. Claims 2-6, 12-14, 17, 67, 68, and 72 fall with claim 1. *See* 37 C.F.R. § 41.37(c)(1)(vii).

OBVIOUSNESS -- CLAIMS 7, 8, AND 10

ISSUE

Claims 7, 8, and 10 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, Mackey, and Anderson (Ans. 8-9). The claims read as follows:

7. The method of claim 1, wherein the sample of target genomic nucleic acid is prepared using a procedure selected from the group consisting of random priming, nick translation, and amplification, of a sample of genomic nucleic acid to generate segments of target genomic nucleic acid; followed by a step comprising fragmentation or enzymatic digestion, or both, of the segments to generate a sample of target genomic nucleic acid consisting of sizes smaller than 200 bases.

8. The method of claim 7, wherein the random priming, nick translation, or amplification, of the sample of genomic nucleic acid to generate segments of target genomic nucleic acid incorporates detectably labeled base pairs into the segments.

10. The method of claim 1, further comprising prior to step (b), fragmenting the sample of target genomic nucleic acid to sizes smaller than about 200 bases by DNase enzyme digestion.

With respect to claim 7, the Examiner finds that Kallioniemi discloses generating nucleic acids by polymerase chain reaction (PCR), nick translation, or random priming (Ans. 9). Regarding claim 8, the Examiner finds that Kallioniemi teaches labeling nucleic acids by nick translation or random priming (*id.*).

The Examiner concedes, however, that Kallioniemi, McGill, Pollack, and Mackey “do not teach fractionation of DNA by DNase digestion” (Ans. 9). To meet that limitation, the Examiner cites Anderson as teaching

“fragmentation of genomic DNA to sizes below 200 base pairs by digestion with 2.2 ng or more of DNase I” (*id.* (citing Anderson, Figure 1)).

Based on these teachings, the Examiner concludes that a person of ordinary skill in the art would have considered it obvious to use Anderson’s DNase I to fragment the genomic DNA in the method of Kallioniemi, McGill, Pollack, and Mackey (Ans. 9). The Examiner urges that the “motivation to do so, provided by Anderson, would have been that DNase I digestion was sequence-independent and the size[] distribution obtained could be regulated by regulating the amount of DNase I in the reaction” (*id.* (citing Anderson 3019)).

Appellants argue⁴ that claims 7, 8, and 10 depend directly or indirectly from claim 1, and Anderson does not remedy the previously argued deficiencies of Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, and Mackey (App. Br. 11). Moreover, Appellants argue, “because the motivation to combine teachings of the cited references must exist in the cited references themselves, it is improper to use Anderson as the motivation to combine Kallioniemi, McGill, Pollack and Mackey” (*id.* (citing MPEP § 2143.01)).

Appellants further argue that the cited references do not meet the limitations of claims 7, 8, and 10 (App. Br. 12). In particular, Appellants urge, Anderson does not remedy the shortcomings of Kallioniemi, McGill, Pollack, GibcoBRL and Mackey with respect to claims 7, 8, and 10 because Anderson does not teach “fragmentation of target genomic DNA that has

⁴ Appellants argue that claim 7 is “representative” of the claims subject to this rejection (App. Br. 7), but then separately argue that each of the claims is patentable over the cited references (*id.* at 12).

both strands labeled with a detectable moiety. Instead, the agarose gel shown in Figure 1 of Anderson was obtained by digesting lambda DNA with DNase I. No evidence has been provided that both strands of the lambda DNA have been labeled” (*id.*; *see also* Reply Br. 12-13).

In view of the positions advanced by Appellants and the Examiner, the issues with respect to this rejection are whether the Examiner erred in finding that the cited references meet all of the limitations of claims 7, 8, and 10, and whether the Examiner erred in finding that the cited references would have prompted a person of ordinary skill in the art to practice the methods recited in the claims.

FINDINGS OF FACT

22. Kallioniemi discloses a fluorescent in-situ hybridization (FISH) experiment in which a platelet derived growth factor beta (PDGFB) probe is hybridized to an array of immobilized nucleic acids from a number of different tumors (Kallioniemi [0113]-[0119] (Example 6)). Kallioniemi discloses that the PDFGB probe was obtained by PCR screening of a genomic library (*id.* at [0114]).

23. Kallioniemi discloses that the PDGFB probe was “labeled by nick-translation or random priming with SpectrumOrange dUTP” (Kallioniemi [0116]).

24. Anderson discloses a DNA sequencing method in which the molecule to be sequenced “is first subjected to limited attack by a non-specific endonuclease (DNase I in the presence of Mn^{++}), fractionated by size and cloned in a single-stranded phage vector. Clones are then picked at random and used to provide a template for sequencing by the dideoxynucleotide chain termination method” (Anderson 3015).

25. To determine whether DNase I digestion was useful for producing random fragments from larger DNA molecules, Anderson digested lambda DNA and found that “[t]he average sizes of the resultant fragments were found to be inversely proportional to the amount of enzyme in the digest and the distributions were smooth and unimodal (Fig. 1). This indicated that there were no obvious preferentially digested or resistant sequences present in the digest” (Anderson 3019).

26. For sequencing purposes Anderson used a DNase I concentration that resulted in fragments with a median size of 300-500 base pairs (Anderson 3015).

ANALYSIS

Appellants’ arguments do not persuade us that the Examiner erred in finding that the cited references meet all of the limitations of claims 7, 8, and 10. Nor are we persuaded that the Examiner erred in finding that the cited references would have prompted a person of ordinary skill in the art to practice the methods recited in the claims.

Claim 7 recites the method of claim 1, with the additional steps of preparing the genomic nucleic acid sample by random priming, nick translation, or amplification, and then reducing the sample to nucleic acid fragments of less than 200 bases by either enzymatic digestion or fragmentation. Claim 8 requires that the random priming, nick translation, or amplification results in incorporating detectably labeled base pairs into the segments.

Kallioniemi discloses that a DNA fragment to be hybridized to an immobilized array can be prepared by PCR (amplification), followed by labeling using random priming or nick translation (FF 22, 23). We therefore

agree with the Examiner that Kallioniemi suggests using those processes to prepare the nucleic acid samples to be hybridized to Kallioniemi's genomic CGH array (*see* FF 2), as recited in claims 7 and 8.

Regarding the enzymatic digestion of the nucleic acid samples recited in claims 7 and 10, Anderson evidences that DNase I was known in the art to be useful to generate random fragments of a desired size when used at an appropriate concentration (FF 24, 25). Thus, taken as a whole, a person of ordinary skill in the art performing Kallioniemi's array-based CGH hybridization would have been advised that (a) smaller fragments were advantageous in array-based CGH-type hybridizations (Pollack (FF 18)), (b) that 20 base fragments provided stable and selective hybridizations (McGill (FF 14)), and (c) that DNase I was effective in predictably reducing the size of nucleic acids in a random fashion (Anderson (FF 24, 25)).

In view of these disclosures, we agree with the Examiner that an ordinary artisan practicing Kallioniemi's CGH hybridization, aware of the suitability of DNase I as a means for predictably reducing the size of nucleic acids, would have been prompted to use DNase I to prepare the sample fragments in the sizes suggested by Pollack and McGill. We are therefore not persuaded that the Examiner erred in finding that the cited references meet all of the limitations of claims 7, 8, and 10, nor do we agree that the Examiner erred in finding that the cited references would have prompted a person of ordinary skill in the art to practice the methods recited in claims 7, 8, and 10.

We therefore affirm the Examiner's obviousness rejection of claims 7, 8, and 10.

OBVIOUSNESS -- CLAIM 9

ISSUE

Claim 9 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, Mackey, Anderson, and Waggoner (Ans. 9-10).

Claim 9 recites “[t]he method of claim 8, wherein the detectable label comprises Cy3TM or Cy5TM.”

The Examiner cites Waggoner as disclosing the dyes of claim 9 for use in labeling nucleic acids (*id.* at 10). The Examiner concludes that a person of ordinary skill in the art would have considered it obvious to use Waggoner’s dyes in Kallioniemi’s methods in view of Waggoner’s disclosure that “cyanine dyes were used for detecting mixtures of components because they had a wide range of excitation and emission wavelengths” (*id.* (citing Waggoner, col. 4, ll. 36-49)).

Appellants argue that claim 9 depends indirectly from claim 1, and that claim 9 is therefore unobvious in view of the previously argued deficiencies of Kallioniemi and the other references (App. Br. 12-13). Moreover, Appellants argue, “because the desirability of combining cited references must exist in the cited references themselves, it is improper to use Anderson as the motivation to combine Kallioniemi, McGill, Pollack and Mackey” (*id.* at 13 (citing MPEP § 2143.01)).

Appellants further argue that Waggoner does not remedy the shortcomings of the other references with respect to claim 9 because “Waggoner does not disclose labeling of both strands of genomic DNA and fragmentation or enzymatic digestion of the genomic DNA. Therefore,

Waggoner's disclosure of Cy3 and Cy5 does not render claim 9 (Group III) obvious” (*id.*; *see also* Reply Br. 13).

In view of the positions advanced by Appellants and the Examiner, the issue with respect to this rejection is whether the Examiner erred in finding that the cited references would have prompted a person of ordinary skill in the art to practice the method recited in claim 9.

FINDINGS OF FACT

27. Waggoner discloses “luminescent polymethine cyanine and related polymethine dyes” useful in labeling “proteins and other materials, including nucleic acids [such as] DNA” (Waggoner, col. 2, ll. 58-61) and “derivatized nucleotides” (*id.* at col. 11, ll. 15-16).

28. Waggoner discloses that the dye can be used to “form a dye conjugated DNA or RNA fragment which is then directed to a main strand of DNA or RNA to which the piece is complementary. The same test method can be employed to detect the presence of any complementary main strand of DNA” (Waggoner, col. 4, ll. 31-35).

29. Appellants do not dispute that Waggoner’s dyes include the Cy3 and Cy5 recited in claim 9.

ANALYSIS

Appellants’ arguments do not persuade us that the Examiner erred in finding that the cited references would have prompted a person of ordinary skill in the art to practice the method recited in claim 9. Claim 9 limits the process recited in claim 8 to one in which the detectable label incorporated into the target genomic DNA is Cy3 or Cy5.

As the Examiner points out, and Appellants do not dispute, Waggoner discloses that those dyes are suitable for use in labeling nucleic acids,

including in hybridization experiments (FF 27-29). Moreover, Pollack discloses the use of those dyes in a random-primer labeling of sample used in a cDNA array-based CGH method (FF 17).

In view of these disclosures, we agree with the Examiner that a person of ordinary skill in the art practicing Kallioniemi's CGH hybridization, aware of the suitability of Cy3 and Cy5 as labels in nucleic acid hybridization experiments, including CGH-type methods, would have been prompted to use those dyes in Kallioniemi's hybridizations.

It may be true that Waggoner does not disclose using the dyes in methods in which the DNA fragments are produced enzymatically. However, as discussed above, the desirability of performing the claimed steps is suggested by the other references, whereas Waggoner is applied to show that the claimed dyes were known to be suitable in the type of process claimed.

Thus, "[n]on-obviousness cannot be established by attacking references individually where the rejection is based upon the teachings of a combination of references. . . . [The reference] must be read, not in isolation, but for what it fairly teaches in combination with the prior art as a whole." *In re Merck & Co., Inc.*, 800 F.2d 1091, 1097 (Fed. Cir. 1986).

We affirm the Examiner's obviousness rejection of claim 9.

OBVIOUSNESS -- CLAIM 11

Claim 11 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, Mackey, Anderson, and Ordahl (Ans. 10-12).

Claim 11 recites "[t]he method of claim 1, further comprising prior to step (b), fragmenting the sample of target genomic nucleic acid to sizes

smaller than about 200 bases by applying shearing forces sufficient to fragment genomic DNA followed by DNase enzyme digestion of the sheared DNA.”

The Examiner cites Ordahl as disclosing the shearing method recited in claim 11 for use in fragmenting genomic DNA, and Anderson for its use of DNase I in fragmenting genomic DNA (*id.* at 11).

Based on these teachings the Examiner concludes that a person of ordinary skill in the art would have considered it obvious to use Ordahl’s shearing and Anderson’s enzymatic digestion to fragment the genomic target DNA in Kallioniemi’s methods, based on Ordahl’s disclosure that “it was advantageous to use short DNA fragments in hybridization” (*id.* (citing Ordahl, 2885)), and Anderson’s disclosure that “DNase I digestion was sequence-independent and the sizes distribution obtained could be regulated by regulating the amount of DNase I in the reaction” (*id.* at 12 (citing Anderson 3019)).

Appellants argue that claim 11 depends from claim 1, and that claim 11 is therefore unobvious in view of the previously argued deficiencies of Kallioniemi and the other references (App. Br. 13). Moreover, Appellants argue, “Ordahl discloses a technique that produces DNA fragments of approximately 230 base pairs, whereas claim 11 recites fragmenting to produce sizes smaller than about 200 bases by shearing followed by enzymatic digestion of the sheared DNA with DNase” (*id.* at 14).

Appellants further argue that “Anderson’s disclosure of digesting lambda DNA with DNase I is not sufficient, because there is no disclosure, teaching or suggestion that both strands of the lambda DNA have been labeled,” and that therefore “no proper evidence has been provided that the

combination of the citations necessarily discloses all elements that are recited in claim 11” (*id.*; *see also* Reply Br. 14).

In view of the positions advanced by Appellants and the Examiner, the issue with respect to this rejection is whether the Examiner erred in finding that the cited references would have prompted a person of ordinary skill in the art to practice the method recited in claim 11.

FINDINGS OF FACT

30. Ordahl discloses that “DNA reassociation and hybridization analysis requires the use of fragmented DNA. . . . In general, . . . on kinetic and other grounds it is advantageous to use short DNA fragments less than 500 base pairs in length” (Ordahl 2985).

31. Ordahl discloses that “DNA fragmented by conventional French press shearing procedures (30,000 lbs/in²) has a number-average fragment size of 230 base pairs” (*id.*).

ANALYSIS

Appellants’ arguments do not persuade us that the Examiner erred in finding that the cited references would have prompted a person of ordinary skill in the art to practice the method recited in claim 11. We note, as Appellants argue, that Ordahl discloses preparing DNA fragments 230 bases long, whereas the claims require the fragments to be less than 200 base pairs. It may also be true that Anderson does not disclose labeling its DNase digested fragments.

However, Ordahl and Anderson are not cited as the sole evidence of obviousness, but are instead cited in combination with other references. Thus, Appellants’ arguments regarding the shortcomings of Ordahl and

Anderson by themselves are unavailing with respect to the rejection at issue. *See In re Merck*, 800 F.2d at 1097.

As discussed above, Kallioniemi discloses a CGH method in which a genomic DNA sample of interest is hybridized to an array. McGill discloses that fragments as small as 20 base pairs are suitable in hybridizations. Ordahl (FF 30, 31) and Anderson (FF 24-26) disclose that shearing and DNase I digestion, respectively, are predictable methods of reducing genomic DNA to smaller fragments. Given these disclosures, we agree with the Examiner that a person of ordinary skill in the art practicing Kallioniemi's CGH method would have been prompted to use known methods, including shearing and DNase I digestion, to prepare genomic DNA fragments of a size suitable for the hybridization process.

We therefore also agree with the Examiner that claim 11 would have been obvious to a person of ordinary skill in the art, and affirm the Examiner's obviousness rejection of that claim.

SUMMARY

We affirm the Examiner's rejection of claims 1-6, 12-14, 17, 67, 68, and 72 under 35 U.S.C. § 103(a) as being unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, and Mackey.

We affirm the Examiner's rejection of claims 7, 8, and 10 under 35 U.S.C. § 103(a) as being unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, Mackey, and Anderson.

We affirm the Examiner's rejection of claim 9 under 35 U.S.C. § 103(a) as being unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, Mackey, Anderson, and Waggoner.

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We affirm the Examiner's rejection of claim 11 under 35 U.S.C. § 103(a) as being unpatentable over Kallioniemi, McGill, Pollack, the GibcoBRL Catalog, Mackey, Anderson, and Ordahl.

TIME PERIOD

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

cdc

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